PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of:

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For: LANE, David

Examiner: Davis, Minh-Tam

Art Unit: 1642

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DECLARATION UNDER 37 CFR' 37 C.F.R. 1.132

Assistant Commissioner for Patents
Washington, DC 20231

SIR:

I Professor Karen Vousden, hereby declare and say as follows:

1. I received my Ph.D. in Genetics from the University of London. I carried out postdoctoral fellowships with Professor Chris Marshall at the Institute of Cancer Research in London and Dr. Douglas Lowy at NCI before becoming Head of the Human Papillomavirus Group at the Ludwig Institute for Cancer Research in London in 1987.

2. After joining the ABL-Basic Research Program as Head of the Molecular Carcinogenesis Section in 1995, I was appointed Director of the Molecular Virology and Carcinogenesis Laboratory in 1997 and Interim Director of the ABL-Basic Research Program in 1998. I was then appointed Chief of the Regulation of Cell Growth Laboratory (RCGL), Division of Basic Sciences, NCI.

3. I am currently the Director of the Cancer Research UK (CRUK) Beatson Institute, Garscube Estate, Switchback Road, Bearsden, Glasgow, UK.

4. I have pursued a distinguished career on both sides of the Atlantic untangling the molecular mechanisms that underlie cancer. I have focused on a number of proteins, in particular p53, which act as cancer tumour suppressors in normal cells, but whose functions are disrupted in most human cancers. My work has led to the recognition of some key features of p53 that highlight the importance of apoptosis, or cell death, in the tumour suppressor function of p53. My studies have contributed to the realization that tumour cells have a deregulated pathway for RB (a protein critical for cell cycle regulation) and are more sensitive to p53 driven cell-death than their normal counterparts. Of particular importance is the pivotal work in identifying the role of the Mdm2 protein in regulating p53 activity, which has opened a path to possible re-activation of p53 in some tumour cells, which could provide

a beneficial and therapeutic effect in the treatment of cancer. Recent and selected publications I have authored or co-authored include:

Carter S, Bischof O, Dejean, Vousden KH. (2007). C-terminal modifications regulate MDM2 dissociation and nuclear export of p53. *Nature Cell Biology* 9, 428-435.

Uldrijan S, Pannekoek WJ, Vousden KH. (2007). An essential function of the extreme C-terminus of MDM2 can be provided by MDMX. *EMBO Journal* 26, 102-112.

Wilson JM, Henderson G, Black F, Sutherland A, Ludwig RL, Vousden KH, Robins DJ. (2007). Synthesis of 5-deazaflavin derivatives and their activation of p53 in cells. *Bioorganic & Medicinal Chemistry* 15, 77-86.

Bensaad K, Tsuruta A, Selak MA, Vidal MNC, Nakano K, Bartrons R, Gottlieb E, Vousden KH. (2006). TIGAR, a p53-inducible regulator of glycolysis and apoptosis. *Cell* 126, 107-120.

Vousden KH. (2006). Outcomes of p53 activation – spoilt for choice. *Journal of Cell Science* 119, 5015-20.

Bensaad K, Vousden KH. (2005). Saviour and Slayer: the two faces of p53. *Nature Medicine* 11, 1278-1279.

Fogal V, Kartasheva N, Trigiante G, Llanos S, Yap D, Vousden KH, Lu X. (2005). ASPP1 and ASPP2 are new transcriptional targets of E2F. *Cell Death Diff.* 12, 369-376.

Rossi M, De Laurenzi V, Munarriz E, Green DR, Liu YC, Vousden KH, Cesareni G, Melino G. (2005). The ubiquitin-protein ligase itch regulates p73 stability. *EMBO J.* 24, 836-848.

Vousden KH. (2005). Apoptosis. p53 and PUMA: a deadly duo. *Science* 309, 1685-1686.

Vousden KH, Prives C. (2005). p53 and prognosis: new insights and further complexity. *Cell* 120, 7-10.

Weber HO, Ludwig, RL, Morrison D, Kotlyarov A, Gaestel M, Vousden KH. (2005). HDM2 phosphorylation by MAPKAP kinase 2. *Oncogene* 24, 1965-1972.

Yang Y, Ludwig RL, Jensen JP, Pierre S, Medaglia MV, Davydov I, Safiran YJ, Oberi P, Kenten J, Phillips AC, Weissman AM, Vousden KH. (2005). Small molecule inhibitors of HDM3 ubiquitin ligase activity stabilize and activate p53 in cells. *Cancer Cell* 7, 547-559.

Yee KS, Vousden KH. (2005). Complicating the complexity of p53. *Carcinogenesis* 26, 1317-1322.

5. I have read and understand McCann et al, British Journal of Cancer (1995) 71, 981-985; Bottger et al 1996, 1996 (Oncogene, 13: 2141-2147) and the comments made by the examiner in the Advisory Action dated 13 December 2006.

6. At the time of publication of the McCann paper, it was understood that inhibition of p53 function is important for the development of many cancers. It was also understood that this might be the consequence of a number of different events such as (but not limited to)

1. Mutation within the p53 gene.
2. Over-expression of Mdm2 – a known negative regulator of p53.
3. Expression of the human papillomavirus E6 protein

7. There was evidence that these alterations are mostly mutually exclusive. In other words, tumours with E6 or Mdm2 over-expression do not have mutated p53 and *vice versa.* The understanding was that it is only necessary to inactivate p53 through one mechanism. (Crook et al, Oncogene 6:873-875, 1991; Scheffner et al, PNAS 88: 5523-5527, 1991; Crook et al, Lancet 339: 1070-1073, 1992; Leach et al, Cancer Res 53: 2231-2234, 1993; Oliner et al., Nature 358: 80-83, 1992).

8. At the time of filing US09/403,440 I was aware that (i) p53 binds Mdm2; (ii) Mdm2 inhibits p53 activity; (iii) inhibition of Mdm2 in normal cells will activate p53; and (iv) Mdm2 is over-expressed in some tumours and this if often associated with retention of wild type p53.

9. Two papers were published in 1995 (Jones et al, Nature 378:206-208, 1995; and Montes de Oca Luna et al, Nature 378:203-206, 1995) that showed that deletion of Mdm2 in mice causes embryonic lethality owing to the activation of p53. Accordingly, I was aware that inhibition of Mdm2 can cause activation of p53 in cells where Mdm2 levels are normal (i.e. not over-expressed) but that this was very deleterious to normal tissue. These findings suggested to me that a therapy to inhibit mdm2/p53 would not be selective for tumours where Mdm2 is expressed at normal levels. Instead, the findings suggested that such a therapy could well be non-specifically toxic and consequently would not be a good approach for tumours without Mdm2 over-expression.

10. Further, at the time of filing US09/403,440, I was aware that some tumours show over-expression of Mdm2. It was expected that these tumour cells would be more sensitive to Mdm2 inhibition than normal cells, so the inhibition of Mdm2 could be useful as a cancer therapy specifically in these cases.

11. It was assumed at that time that in tumours with no over-expression of Mdm2, p53 was inhibited through other, unknown mechanisms. Consequently, it was not known at the time whether inhibition of Mdm2:p53 interaction in such tumours would be an effective therapy, and indeed, there was evidence that such an approach could be very deleterious to normal tissues.

12. Before publication of the McCann paper, it was not clear how many tumour types would show frequent over-expression of Mdm2. The McCann paper examines this question in breast cancers. They look at both gene amplification and protein expression and find that only 7% of these cancers show over-expressed Mdm2. The conclusion from these studies is that inactivation of p53 as a consequence of Mdm2 over-expression occurs in only few breast cancers. As stated by McCann et al *"We conclude that MDM2 gene amplification occurs at a lower frequency in breast cancer than in non-epithelial tumours."* (Summary, lines 8/9)

13. As stated above, at the time of the McCann paper, it was held that inhibition of the p53/Mdm2 interaction would only be effective in cancers that over-express Mdm2. Therefore the McCann paper would teach that this type of therapy would be effective in only a small proportion of breast cancers. There is nothing in this paper to suggest that disruption of the p53/Mdm2 interaction would be effective in cancers in which Mdm2 is not over-expressed.

14. McCann et al also examine p53 expression. They show that, as expected, over-expression of Mdm2 is associated with low levels of p53. It is important to realise that low p53 levels are indicative of the retention of wild type p53 – so this study supports the suggestion made earlier that over-expression of Mdm2 can inhibit p53 - and therefore remove the requirement for a mutation within p53. However, the study suggests that this correlation is not complete, and in some cancers alterations in both Mdm2 and p53 may have occurred.

15. The examiner is incorrect in her view that McCann et al teach that *in cancers which do not express mdm2, such as breast cancer cells, the protein expression of mdm2 is significantly associated with low levels of p53.* The study by McCann et al shows that although most breast cancers do not over-express Mdm2, a few of them do show elevated Mdm2 expression, and these tumours are significantly associated with low (i.e. wild type) p53 levels. McCann et al state that *"at the protein level, MDM2+ tumours were significantly associated with tumours having low levels of p53 staining".* (Summary, lines 7/8) This means that those few breast cancers that over-express Mdm2 tend to show low levels of p53 – indicating a retention of wild type p53.

16. Taking McCann et al together with the study from Bottger, and without the knowledge of the invention claimed in the present application, I would conclude that a therapy based on the 12 amino acid peptide would only be expected to be effective in 7% of breast cancers (i.e. those with over-expressed Mdm2) and would suggest that most breast cancers would not benefit from such therapy.

17. At the time of the McCann et al paper it would have been reasonable to assume that in cells where low or normal levels of Mdm2 exist, inactivation of the p53 pathway to allow aberrant tumour growth would have arisen from another mechanism.

18. For the reasons set forth in paragraphs 6 through 17 of this Declaration, I believe the results disclosed in US09/403,440 where they have shown that inhibition of Mdm2:p53 has a growth reducing effect in tumour cells in which Mdm2 is not over-expressed and consequently is a useful therapy for these cells was surprising given the understanding of the mechanisms involved in p53 function at the time US09/403,440 was filed.

19. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information or belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

Signed _____ on 27-06-07